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Research Paper

Influence of dimethyl sulfoxide used as a solvent on the physical properties and long-term dentin bonding of hydrophilic resins



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ABSTRACT

Objective: To examine the feasibility of dimethyl sulfoxide (DMSO) incorporation into relatively hydrophilic resins as a new potential method to improve the durability of resin–dentin bonds.

Methods: Six experimental light-curing BisGMA/HEMA resins solvated in ethanol and DMSO with increasing concentrations of DMSO (0, 0.5, 1, 2, 4 and 10 wt%) were prepared. The degree of conversion (DC) was evaluated by Fourier Transform Infrared Spectroscopy ($n=8$); water sorption (Wsp) and water solubility (Wso) were gravimetrically assessed ($n=10$); and flexural strength (FS) and elastic modulus (E) were determined by a three-point bending flexural test ($n=10$). Flat dentin surfaces on sound third molars ($n=10$ /group) were bonded with resins containing 0, 2, 4 and 10 wt% DMSO used as a two-step etch-and-rinse system. Dentin microtensile bond strength was determined at 24 h and after two-year aging in artificial saliva at 37 °C.

Results: DMSO significantly affected Wsp ($p=0.0006$), DC, Wso, FS, and E ($p<0.0001$). In

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general, the resins' mechanical/physical properties were not affected by 2% or lower DMSO incorporation. Incorporation of 4% or higher DMSO content significantly increased DC, W_{sp} and W_{so}, but 2% or higher DMSO concentrations significantly reduced FS and E. No influence on immediate dentin bond strength occurred up to 4% DMSO incorporation. While 4% or higher DMSO concentrations impaired bond strength over time, the resin containing 2% DMSO presented significant higher dentin bond strength compared to the control resin after two year-aging.

Significance: The use of DMSO as a new solvent in adhesive dentistry improves dentin bonding of relatively hydrophilic resins over time. 2% DMSO incorporation in BisGMA/HEMA resins should be sufficient to reduce bond strength loss without compromising polymer mechanical strength and physical properties.

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1. Introduction

During the last decade, the understanding of mechanisms involved in the degradation of dentin–adhesive interfaces has substantially advanced (Tjäderhane et al., 2013a, 2013b, 2014) and measures to improve dentin bond durability have become one of the main research topics in adhesive dentistry (Tjäderhane et al., 2013a, 2013b, 2014; Frassetto et al., 2015; Liu et al., 2011; Carvalho et al., 2012, 2003). A cascade of events (i.e., hydrolysis of both collagen matrix and the resin constituents) invariably contributes to hybrid layer degradation resulting in premature failure of the adhesive interface (Tjäderhane et al., 2013a, 2013b, 2014). For this reason, stable polymers presenting low water sorption/solubility with high mechanical strength should be formed (Carrilho et al., 2005; Cho et al., 2005; Malacarne et al., 2006) at the resin–dentin bonded interface in order to better resist hydrolytic degradation.

Even though adhesives systems have been criticized for quite some time as being too hydrophilic (Tay and Pashley, 2003), the etch-and-rinse bonding mechanism still relies on relatively hydrophilic monomers for bonding hydrated dentin substrate in clinically relevant protocols. Current dental adhesives are essentially comonomer blends solvated in volatile organic solvents. Solvents dilute viscous monomers, displace water and act as vehicle in the transportation of methacrylate monomers into demineralized dentin allowing better monomer–tissue interactions (Ekambaram et al., 2015). In this regard, not only solvent type but also solvent–monomer ratios are critical factors in resin–dentin bonding (Carvalho et al., 2003; Ekambaram et al., 2015). Most attempts to increase the stability of resin–dentin bonds have mainly focused on prevention of collagen degradation (Tjäderhane et al., 2014; Frassetto et al., 2015; Liu et al., 2011), improvement of collagen hybridization (Tjäderhane et al., 2013a, 2013b; Frassetto et al., 2015; Liu et al., 2011) or collagen remineraliation (Tjäderhane et al., 2013a, 2013b; Toledano et al., 2015). User friendly methods aiming to simultaneously address such dentin bonding limitations are still necessary.

Recently, dimethyl sulfoxide (DMSO; [(CH₃)₂SO]) was introduced in adhesive dentistry as a new solvent capable to improve dentin bonding (Tjäderhane et al., 2013a, 2013b;

Stape et al., 2015). DMSO is a polar aprotic solvent that dissolves both polar and non-polar compounds. It is a poly-functional molecule, with a highly polar S=O group and two hydrophobic methyl groups, fully miscible in most solvents and in hydrophilic and hydrophobic monomers used in adhesive dentistry (Geurtsen et al., 1998). Due to its small size and amphiphilic nature, DMSO efficiently penetrates biological surfaces, which makes it perhaps the best currently known penetration enhancer for medical purposes (Marren, 2011). DMSO has the ability to dissociate the highly cross-linked collagen into a sparser network of apparent fibrils (Zimmerley et al., 2009), break water self-associative tendency (Vishnyakov, 2001) and consequently improve the wettability of demineralized dentin (Mehtälä et al., 2010). Dentin pretreatment with low DMSO concentrations improves long-term bonding (Tjäderhane et al., 2013a, 2013b) at the expense of an extra step in the etch-and-rinse bonding technique. Therefore, solvation of adhesive monomers by DMSO could possibly benefit dentin bonding in a similar manner, but without the need of an extra bonding step, as long as the resin mechanical/physical properties would not be compromised.

The aim of this study was to assess the use of DMSO as a new potential dental adhesive solvent to be incorporated in relatively hydrophilic resins. The mechanical/physical properties, including degree of conversion, water sorption, water solubility, flexural strength, and elastic modulus of six experimental two-step total-etch adhesive resins with increasing concentrations (0, 0.5, 1, 2, 4, and 10 wt%) of DMSO were evaluated. The effect of adhesive resins containing different DMSO concentrations on the immediate and long-term dentin microtensile bond strength was also investigated to determine if DMSO incorporation in two-step total-etch adhesive resins would influence dentin bond strength. The null hypotheses to be tested were as follows: (i) DMSO incorporation in experimental hydrophilic resins would have no effect on degree of conversion, water sorption, water solubility, flexural strength, and elastic modulus; and (ii) adhesive resins containing DMSO would not affect immediate and long-term dentin bond strength.

2. Materials and methods

2.1. Experimental adhesive system composition

A neat light-curing hydrophilic resin-blend containing 56% (w/w) 2,2-bis[4-(2-hydroxy-3-methacryloyloxypropoxy)]-phenylpropane (BisGMA), 28.65% 2-hydroxyethyl methacrylate (HEMA), 0.25% camphorquinone (CQ), 1% 2-ethyl-4-aminobenzoate (EDMAB), and 0.1% butylhydroxytoluene (BHT) was produced. The neat resin-blend was solvated either in 20% ethanol (control) or replacing ethanol partially with increasing DMSO concentrations to produce six experimental bonding resins containing a gross w/w % of 0%, 0.5%, 1%, 2%, 4%, and 10% DMSO. A pilot study was previously performed to determine the minimum gross amount of solvent to be incorporated in the resin blends to produce immediate dentin bonding values within an acceptable range. The total amount of solvent, either ethanol (control) or ethanol/DMSO mixtures, remained 20% in all adhesive resins. All components were obtained from Sigma-Aldrich, St. Louis, MO, USA.

2.2. Degree of conversion

Absorption spectra of uncured and cured experimental adhesives were obtained by Fourier Transform Infrared Spectroscopy (Spectrum 100 Optica; PerkinElmer, MA, USA) equipped with a HeNe laser recorded in the region between 2000–1000 cm^{-1} , with 16 scans, at 4 cm^{-1} spectral resolution using a baseline method (Rueggeberg et al., 1990). Monomer conversion was determined by measuring the decrease of the C=C rationed before and after polymerization to an internal aromatic C=C standard. A circumferential (3 mm \times 0.8 mm) silicon hollow mold was centralized over the ATR crystal surface and a 5 μL drop of each experimental adhesive was placed inside the mold in direct contact with the ATR crystal. A piece of mylar strip was immediately placed over the top of the deposited resin to exclude oxygen and prevent solvent evaporation. Photoactivation was performed at a fixed 1 mm tip distance. Infrared spectra were collected after polymerization with a polywave light-emitting diode (LED) light-curing unit (Bluephase 20i, Ivoclar Vivadent, Schaan, Liechtenstein) in high power mode with an output of 1200 mW/cm^2 for 20 s. The light intensity was checked regularly with the accompanying Bluephase meter (Ivoclar Vivadent). After the photocuring exposure, post-cure polymerization was allowed to continue up to 180 s from light initiation and the absorption spectrum was collected for each sample ($n=8$)/group. The average of three readings was considered to obtain the ratio of aliphatic/aromatic peaks for uncured adhesives. Degree of conversion (DC) was calculated by changes in C=C absorption peak ratios of aliphatic (1638 cm^{-1}) and aromatic (1608 cm^{-1}) peaks in both uncured and cured states obtained from the infrared spectra according to the following equation:

$$\text{DC}(\%) = \left(1 - \frac{R^{(\text{Cured})}}{R^{(\text{Uncured})}} \right) \times 100$$

where “R” is the ratio of aliphatic and aromatic peak intensities at 1638 cm^{-1} and 1608 cm^{-1} in cured and uncured adhesives.

2.3. Water sorption and water solubility

The experimental adhesives were poured into customized cylindrical stainless steel molds (0.5 mm thick; 9 mm in diameter) and a Mylar strip was immediately placed on top of it followed by a glass slide before light-polymerization (Bluephase 20i, Ivoclar Vivadent) in high power mode with an output of 1200 mW/cm^2 for 20 s. The polymerized samples for each experimental adhesive ($n=10$) were stored at room temperature for 24 h in dark. Samples were then stored in a desiccator containing dried silica gel at 37 $^{\circ}\text{C}$ for 24 h and weighed with a calibrated digital balance (resolution of 0.01 mg). This drying/weighting cycle was repeated daily until a constant weight (M1) for each specimen was obtained. Constant weight was considered when mass variation was inferior to 0.1 mg in a 24 h period. After drying, the specimens were immersed in distilled water at 37 $^{\circ}\text{C}$ for 7 days. Then, they were, blotted dry to remove visible water on the specimen surface, weighed (M2) and placed back in a desiccator containing dried silica gel at 37 $^{\circ}\text{C}$ until a constant weight was achieved (M3). The values for water sorption (Wsp) and solubility (Wso) were calculated as follows:

$$W_{sp} = \frac{M2 - M3}{V}, W_{so} = \frac{M1 - M3}{V}$$

where M1 is the constant initial mass (μg) of the specimen; M2 is the mass (μg) of the specimen after immersion in water for 7 days; M3 is the constant mass (μg) of specimen after removal from water and drying; and V is the volume (mm^3) of the specimen.

2.4. Flexural strength and elastic modulus

The adhesive resin specimens were fabricated in customized stainless steel molds according to ISO 4049 specifications except for specimen size. Bar-shaped specimens (25 \times 2 \times 2 mm^3) were prepared for each group ($n=10$). The dental adhesives were placed into a customized stainless steel mold and a Mylar strip was positioned on top of it, followed by a glass slide. Each specimen was light-cured (Bluephase 20i, Ivoclar Vivadent) in high power mode with an output of 1200 mW/cm^2 for 20 s in four overlapping irradiation zones. The specimens were removed from the mold, excess resin from the edges were carefully removed with scalpel blades, and stored in distilled water at 37 $^{\circ}\text{C}$ for 24 h prior to testing. The specimens were tested using three-point bend device (Instron, Instron Inc., Canton, MA, USA) in a mechanical testing machine (Instron 4411, Instron Inc.) at a crosshead speed of 1 mm/min until fracture. The flexural strength (FS) was then calculated using the following equation and expressed in MPa:

$$\text{FS} = \frac{3Fl}{2bh^2}$$

where F is the maximum load exerted on the specimen in Newton, l is the distance between the supports in mm, b is the width of the specimen in mm, and h is the thickness of the specimen in mm. Elastic modulus, in GPa, was determined (Bluehill Software, Instron Inc.) considering specimen

size and the slope of the linear portion of the load–displacement curve for each specimen tested for FS.

2.5. Microtensile bond strength

Forty sound human third molars were extracted as part of normal clinical treatments with patients' (age 18–26 years) informed consent and approval by the Ethical Committee of the Piracicaba Dental School, University of Campinas, Brazil (Protocol #070/2012). Teeth were cleaned, disinfected for one week in 0.5% chloramine-T solution at 4 °C, and stored in distilled water at 4 °C for up to one month before use. A flat coronal dentin surface was obtained by sectioning off the occlusal one-third of the crown (Isomet 1000 Precision Saw, Buehler, Lake Bluff, IL, USA). The smear layer was standardized with 600-grit silicon carbide paper (BuehlerMet, Buehler) for 60 s under water cooling and the specimens were randomly assigned to four groups ($n=10$) according to the selected experimental adhesives for bond strength test containing 0%, 2%, 4% and 10% DMSO.

Bonding procedures were carried out by a single operator in a controlled environment with 24 °C temperature and 45–55% relative humidity. The exposed medium dentin was acid etched for 15 s (Etchant 37% phosphoric acid, 3M ESPE, St. Paul, MN, USA), rinsed with water for 30 s, and blot dried leaving dentin slight moist. Two coats of the selected experimental adhesives were actively applied with a fully saturated brush tip for 10 s, blown dry for 5 s after each coat, and light cured for 20 s. Resin composite build-ups (Z250, shade A2, 3M ESPE) were built on top of the bonded dentin surfaces in two 2-mm increments that were individually light-cured for 20 s (Bluephase 20i, Ivoclar Vivadent) in high power mode with an output of 1200 mW/cm² for 20 s. After storage in distilled water at 37 °C for 24 h, the restored segments were sectioned (Isomet 1000 Precision Saw, Buehler) occluso-gingivally into slabs measuring approximately 0.8 mm and further sectioned into composite-dentin sticks, 0.7 mm² cross-sectional area, in accordance with the “non-trimming” technique (Shono et al., 1999) for bond strength testing. Sticks were randomly divided to be tested at 24 h or after storage in artificial saliva at 37 °C for 2 years. The aged sticks were stored for two years at 37 °C in artificial saliva containing (mmoles/L): CaCl₂ (0.7), MgCl₂ · 6H₂O (0.2), KH₂PO₄ (4.0), KCl (30), NaN₃ (0.3), and HEPES buffer. The storage solution was prepared and changed weekly in accordance with a protocol previously described by Pashley et al. (2004).

Sticks were individually attached to microtensile device (OD03d, ODEME Biotechnology, Luzerna, SC, Brazil) using cyanoacrylate adhesive (Super Bonder, Loctite, SP, Brazil) and tested in tensile forces in a universal testing machine (DL2000, EMIC, São José dos Pinhais, SC, Brazil) at a crosshead speed of 0.5 mm/min until failure. The cross-sectional area of each stick was measured with a digital caliper (Absolute Digimatic, Mitutoyo, Tokyo, Japan) to the nearest 0.01 mm in order to calculate the actual μ TBS in MPa. Bond strengths for each tooth at each testing period were determined by the μ TBS average value of a minimum of 10 sticks/period. Tooth was considered the statistical unit. Sticks with premature failures were recorded as 0 MPa for the statistical analysis. Both surfaces of fractured sticks were observed under a

stereomicroscope (Olympus 220670; Olympus, Tokyo, Japan) with 40x magnification for fracture pattern classification. The fracture modes were classified as follows: cohesive (failure exclusive within dentin or resin composite); adhesive failure (failure at resin/dentin interface); and mixed failure (failure at resin/dentin interface with cohesive failure of the neighboring substrates). For the failure modes that could not be accurately established under the stereomicroscope, the surfaces were examined with a scanning electron microscope (LEO 435 VP; LEO Electron Microscopy Ltd., Cambridge, UK).

2.6. Statistical analysis

Statistical analysis was performed with R (R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria). After confirming the normality, Shapiro–Wilk test, and the homocedasticity of the data, Barlett test, one-way ANOVA was used to evaluate how different DMSO concentrations affected degree of conversion, water sorption, water solubility, flexural strength, and elastic modulus. Differences between groups were calculated using Tukey's *post hoc* test ($\alpha=0.05$). μ TBS data was submitted to Repeated Measures ANOVA followed by Tukey's test ($\alpha=0.05$). For μ TBS data, tooth was considered the statistical unit. Moreover, orthogonal polynomial regression analysis using the aged bond strength data was performed to predict the optimum DMSO concentration to yield the highest bond strength values for the specific resin blend tested. Differences in the number of adhesive failures between the groups within each time point was tested with Krusk–Wallis and Mann–Whitney test, and between the different time points within each group with Friedman and Wilcoxon Signed Rank tests ($\alpha=0.05$).

3. Results

Incorporation of DMSO in relatively hydrophilic resins significantly affected degree of conversion ($p<0.0001$), water sorption ($p=0.0006$), water solubility ($p<0.0001$), flexural strength ($p<0.0001$), and elastic modulus ($p<0.0001$). The results of the tested physical/mechanical properties, degree of conversion, water sorption, water solubility, flexural strength, and elastic modulus, for the resin blends with increasing concentrations of DMSO are summarized in Figs. 1–4, respectively. While no significant differences in degree of conversion between resins with DMSO concentration ranging from 0% to 4% occurred, a significant 13.2% increase ($p<0.05$) was observed in resins containing 10% DMSO compared to the resin without DMSO (Fig. 1). A significant increase of 25% and 82% ($p<0.05$) for water sorption and water solubility respectively were observed when the 10% DMSO resin was compared to the control resin: no significant differences occurred in resins containing 4% or less DMSO (Fig. 2). A general trend towards reduction in flexural strength was observed as DMSO concentration increased (Fig. 3). However, resins containing 2% or less DMSO presented no significant differences compared to the control. Incorporation of 4% and 10% DMSO produced, respectively, significantly 24% and 37% reduction ($p<0.05$) in

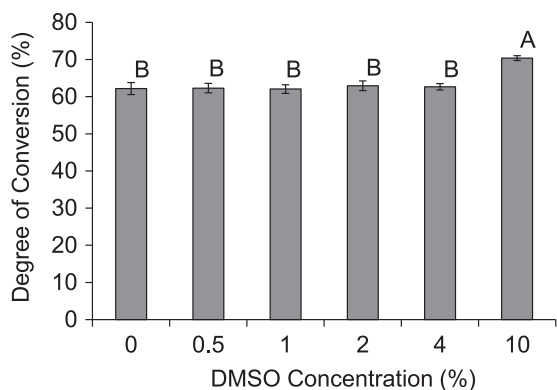


Fig. 1 – Degree of conversion of experimental dental adhesive resins with increasing DMSO concentrations. Mean values and standard deviations ($n=8$)/group. Different letters indicate significant differences ($p<0.05$).

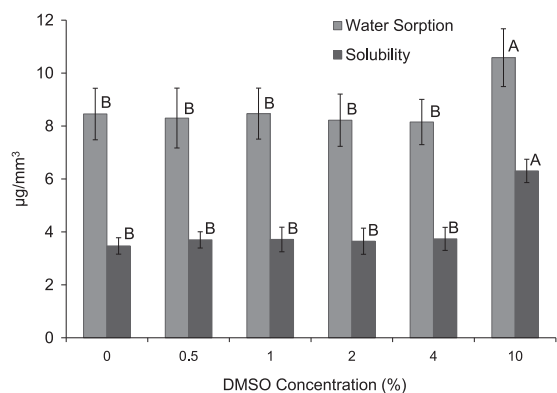


Fig. 2 – Water sorption and solubility of experimental dental adhesive resins containing increasing DMSO concentrations. Mean values and standard deviations ($n=10$)/group. Different letters indicate significant differences ($p<0.05$) for water sorption and solubility.

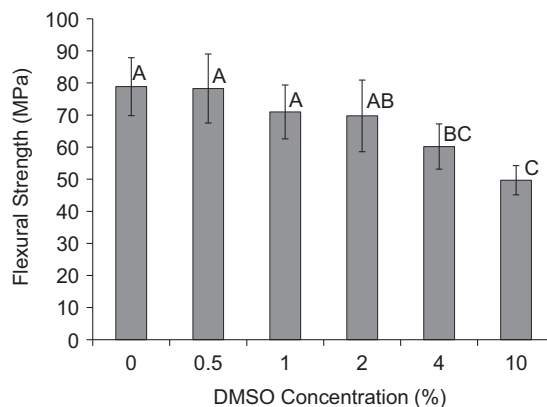


Fig. 3 – Flexural strength of experimental dental adhesive resins with increasing DMSO concentrations. Mean values and standard deviations ($n=10$)/group. Different letters indicate significant differences ($p<0.05$).

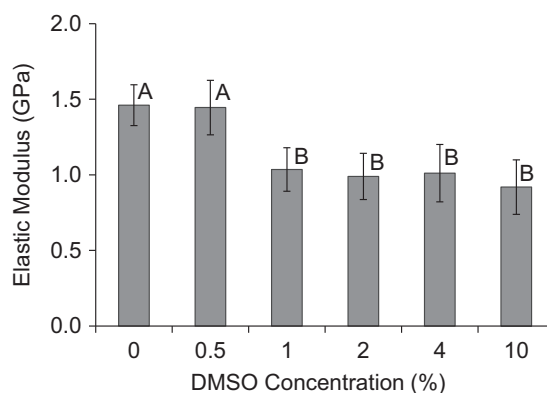


Fig. 4 – Elastic modulus of experimental dental adhesive resins with increasing DMSO concentrations. Mean values and standard deviations ($n=10$)/group. Different letters indicate significant differences ($p<0.05$).

flexural strength when compared to the control resin. Similarly, resin blends containing 1–10% DMSO presented significantly lower ($p<0.05$) elastic modulus than the resin containing 0.5% DMSO and control resin (Fig. 4).

The mean cross-sectional area of tested resin–dentin sticks ($0.71 \text{ mm}^2 \pm 0.11$) ranged from 0.68 to 0.87 mm^2 and no significant differences between groups were detected ($p=0.57$). Microtensile bond strength means (MPa), standard deviations and fracture pattern distribution for all groups are reported in Table 1. Repeated measures ANOVA revealed that “time” ($p<0.0001$) and the interaction between “time” and “adhesive system” ($p=0.0099$) significantly affected dentin microtensile bond strength. At 24 h, no significant differences were detected between the control resin and resins containing 2% and 4% DMSO; however, the resin containing 10% DMSO presented a 22% bond strength reduction compared to the control resin. All tested resins presented significantly lower ($p<0.05$) bond strengths after the 2-year aging. No significant differences were observed between the control and the 4% DMSO resins after aging. Incorporation of 2% DMSO in the hydrophilic resin produced 94% higher dentin bond strengths compared to the control resin and

was significantly higher ($p<0.05$) than any other group after aging. In contrast, incorporation of 10% DMSO significantly reduced ($p<0.05$) the bond strengths over 90% and was practically non-existing after 2 years. Regression analyzes of the aged bond strength values, using a third degree polynomial curve fit to adequately describe the data, estimated 1.58% as the optimum DMSO to produce the highest bond strength values for the tested resin blend (Fig. 5). The tested DMSO concentrations had no impact on immediate failure modes. No significant differences in adhesive failures between the groups were observed at 24 h. A significant increase ($p<0.05$) in adhesive failures after aging compared to 24 h failures occurred in all groups. However, the 2% DMSO resin presented significantly less ($p<0.05$) and 10% DMSO resin had significantly more ($p<0.05$) adhesive failures than any other group (Table 1).

4. Discussion

Since DMSO incorporation in the resins significantly influenced all tested mechanical/physical properties in a concentration

Table 1 – Microtensile bond strength values (MPa) to dentin of relatively hydrophilic resin blends containing increasing mass percentages of DMSO after long-term aging.

	24 h	2 years
0% DMSO	28.17 ^{Aa} ± 5.21 [104 – 11.5 ^{Aa} / 72.1/16.3] (3%)	8.16 ^{Bb} ± 1.51 [101 – 59.4 ^{Bb} / 33.7/6.9] (16%)
2% DMSO	31.75 ^{Aa} ± 5.23 [106 – 10.4 ^{Aa} / 74.5/15.1] (2%)	15.87 ^{Ab} ± 2.8 [105 – 32.4 ^{Ab} / 61.0/6.7] (6%)
4% DMSO	27.73 ^{Aa} ± 5.03 [102 – 11.8 ^{Aa} / 74.5/13.7] (3%)	4.19 ^{Bb} ± 0.81 [104 – 60.6 ^{Bb} / 33.7/5.8] (21%)
10% DMSO	21.91 ^{Ba} ± 3.17 [105 – 19.0 ^{Aa} / 68.6/12.4] (4%)	0.83 ^{Cb} ± 0.1 [102 – 83.3 ^{Cb} / 11.8/4.9] (62%)

Microtensile bond strength (MPa) to dentin and standard deviation ± for all groups. The number of sticks tested per group and the modes of failure are expressed in % into brackets as [number of tested sticks – adhesive/mix/cohesive]. Percentage of premature failures is indicated in parentheses. For bond strength and adhesive failures, same superscripts capital letters indicate no significant differences ($p > 0.05$) in columns and same superscript lowercase letters indicate no significant differences ($p > 0.05$) in rows.

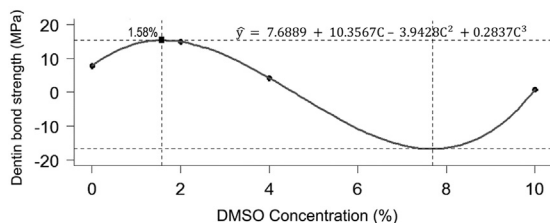


Fig. 5 – Optimum DMSO concentration for the tested resin blends determined by orthogonal regression analysis using a third degree polynomial curve fit. Plotted circle dots represent the aged microtensile bond strength values for each tested DMSO concentration after aging. The square dot represents the optimum DMSO concentration in the tested resin blends to produce the highest bond strength after long-term aging according to the best fit equation.

dependent manner, the first null hypothesis was rejected. To the best of our knowledge, incorporation of DMSO in adhesive resins has not been previously assessed. In the present study, the total mass% of solvents (ethanol and DMSO) was kept constant in all resin blends to avoid a progressive dilution of radicals, physical separation between reactive species during polymer chain formation and reduce oxygen diffusion into the mixture (Holmes et al., 2007). If only increasing aliquots of DMSO were added to the resins, the physical space between the reactive species in the polymeric reaction would increase leading to changes in polymer mechanical/physical properties. So changes in the material properties would be caused by the

amount of solvent and not specifically by the type of solvent used. Similar to commonly used solvents in adhesive dentistry (i.e., acetone, ethanol, water) (Cho et al., 2005; Holmes et al., 2007; Cadenaro et al., 2008; Dickens and Cho, 2005), DMSO concentration played an important role in the mechanical/physical properties of experimental adhesive resins.

BisGMA was the crosslinking monomer used in the tested adhesive resins, which alone or in comonomer mixtures, engages in strong intermolecular hydrogen bonding interactions (Floyd and Dickens, 2006; Lemon et al., 2007). The rigid aromatic core structure in BisGMA excludes the majority of direct intramolecular interactions. Therefore, intermolecular hydrogen bonding occurs mainly between the hydroxyl groups (OH) and the carbonyl groups (C=O) in distinct BisGMA monomers, accounting for the high monomer viscosity observed in BisGMA (Lemon et al., 2007). High viscosity indicates the presence of relatively strong intermolecular interactions which reduce the mobility of monomers during polymerization (Sideridou et al., 2002) and subsequently lower monomer conversion. Solvent incorporation in adhesive systems disrupts such monomer interactions affecting polymer physical/mechanical properties (Cadenaro et al., 2008; Cadenaro, 2012). In general, viscosity plays an important role in monomer conversion (Holmes et al., 2007; Cadenaro et al., 2008; Cadenaro, 2012; Malacarne-Zanon et al., 2009; Dickens et al., 2003). Resin blends with reduced viscosity allow better mobility of the monomer reactive components during photopolymerization most likely improving monomer conversion (Dickens et al., 2003). DMSO is a highly polar aprotic solvent capable of accepting hydrogen bonds, but unable to donate hydrogen bonds. Unlike ethanol that hydrogen bonds to other ethanol molecules (Mizuno et al., 1995), DMSO does not efficiently hydrogen bond to other DMSO molecules. In DMSO–ethanol mixtures, the hydrogen bonding interactions between ethanol and DMSO are relatively weak (Kiefer et al., 2011). Therefore, DMSO/ethanol solutions contain a higher overall number of available molecules to hydrogen bond to BisGMA than pure ethanol. It is difficult to generalize and predict the hydrogen bonding effects on monomer and polymer properties (Lemon et al., 2007). However, we speculate that in DMSO/ethanol mixtures the two nonpolar methyl groups in DMSO can interact with the hydrophobic moieties of distinct BisGMA molecules and increase the overall monomer solubility in the DMSO–ethanol mixture, disrupting monomer intermolecular H-bonding more efficiently than pure ethanol. Moreover, the use of DMSO as a solvent in poly-methacrylate free radical polymerization lowers the termination rates (Gupta and Nandi, 1970). Presumably, DMSO reduced polymer viscosity, increased both polymer chain mobility and the rate of diffusion of radicals, and lowered the termination rates contributing to higher degree of conversion observed with 10% DMSO in the resin.

Nevertheless, increase in degree of conversion does not necessarily reflect the overall quality of the polymer structures (Ye et al., 2007), especially regarding mechanical properties (Ye et al., 2007; Elliott et al., 2001). Monomer crosslinking plays an important role on polymer properties (Ye et al., 2007). The increased degree of conversion of 10% DMSO solvated adhesive resin occurred at the expense of a significant reduction in

flexural strength and elastic modulus. A similar trend was observed with lower DMSO concentration incorporation in the resin blends for flexural strength and elastic modulus. Elastic modulus seemed to be more affected, starting as low as 1% DMSO, while flexural strength presented a significant reduction compared to the control group with 4% DMSO or higher. Whilst higher DMSO concentrations improved degree of conversion, high mass% of DMSO also prevented the approximation between growing polymer chains during polymerization making the crosslinking less likely to occur (Ye et al., 2007). Another important aspect in polymer mechanical strength is the presence of hydrogen bonding between linear chains or covalently crosslinked polymerized networks. Such H-bond interactions can reinforce the three-dimensional polymeric structure producing enhanced mechanical properties (Lemon et al., 2007). Similar to the hydrogen bonding effect between water molecules and polar hydroxyl groups of polymer networks (Ping et al., 2001; Musto et al., 2002), high DMSO content may disrupt such interchain H-bonding locking, altering the molecular structure and increasing polymer chain mobility. It is important to note that the effect of DMSO on flexural strength and elastic modulus was concentration dependent. In general, the outcome produced was resins with significantly reduced flexural strength and elastic modulus as DMSO content increased above 2% and 0.5% DMSO, respectively.

Besides influencing the mechanical properties of resin, monomer crosslinking (Ajithkumar et al., 2000) and H-bonding (Ping et al., 2001; Ito et al., 2005) play also an important role on water sorption/solubility. Reduction in crosslink density increases water sorption in polymer networks (Ajithkumar et al., 2000). The confinement of water clusters in hydrophilic polymers is guided by the strong H-bond interactions between water and the polar groups of polymers either directly or via other water molecules (Ping et al., 2001; Musto et al., 2002). DMSO is a hygroscopic solvent with high hydrogen bonding affinity for water (Kiefer et al., 2011) that may modify H-bonding in water-sorbed polymers containing higher DMSO concentrations. Besides direct H-bonding to hydroxyl groups, unbound DMSO molecules entrapped between molecular chains may alter the dynamic exchange of water molecules between sites and hydration layers due to DMSO capacity to break down water's self-associative tendency (Vishnyakov, 2001). The hydrogen bonding force (δ_H) of DMSO is about only 50% of ethanol [δ_H : 10.2 (J/cm^3)^{1/2} vs 19.4 (J/cm^3)^{1/2}]. Even though ethanol presents higher δ_H , hydrophobic hydration of methyl groups in DMSO manifests a total molar ratio of 1 DMSO:2 H₂O (Vishnyakov, 2001; Kiefer et al., 2011; Dhumal, 2011) or 1 DMSO:3 H₂O (Kiefer et al., 2011; Dhumal, 2011) as the most predominant interactions between DMSO and water. Ethanol has a lower 1 Ethanol:2 Water molar ratio interaction (Liu et al., 2006), so DMSO possesses a higher capacity for water H-bonding than ethanol. Moreover, incorporation of DMSO to adhesive resins certainly increases the overall resin blend polarity, for the polar forces (δ_p) of DMSO and ethanol are 16.4 (J/cm^3)^{1/2} and 8.8 (J/cm^3)^{1/2}, respectively. Since there is a positive correlation of polymer polarity and water sorption (Ito et al., 2005), incorporation of 10% DMSO expedited water sorption when compared to the control group

with reduced polarity. However, resins with 4% DMSO or less presented no significant differences when compared to control group. It should be noted that the increase in water sorption was not caused by reduced conversion within the resin matrices, but most likely due to the presence of a more hygroscopic solvent than ethanol. Nevertheless, the relationship between water sorption and DMSO content in adhesive resins was concentration dependent: 4% or lower DMSO concentrations had no effect on water sorption.

Apart from influencing the mechanical and physical properties of adhesive resins, solvents have a major impact on resin–dentin bonding (Ekambaram et al., 2015). In this sense, the second null hypothesis was rejected since DMSO incorporation in a relatively hydrophilic resin significantly affected dentin bonding. Similar to the mechanical/physical properties, the effect was also concentration dependent. DMSO had no influence on immediate dentin bond strength when 4% or lower concentrations were used, but 2% DMSO produced significant higher bond strengths than any other groups after aging. 10% DMSO significantly impaired both immediate and especially the long-term bond strength. This can be explained by the increased water sorption and solubility since the effect on flexural strength and elastic modulus were less dramatic compared to other DMSO concentration.

Ideally, an adhesive system should contain solvents that facilitate resin penetration within the interfibrillar spaces in demineralized dentin. Since hydrogen bonding in resins significantly affect the surface wetting behavior of monomers and the hydrophilic character associated with the corresponding polymers (Lemon et al., 2007), modification of the resin H-bonding caused by DMSO incorporation most likely altered bonding of the relatively hydrophilic resins to dentin. Although no improvements were observed at 24 h by the microtensile test, enhanced dentin bond strengths were obtained after aging when 2% DMSO was incorporated in the resin blend. DMSO capacity to displace water molecules and to break water self-associative tendency, (Vishnyakov, 2001), certainly played an important role enhancing the adhesive–dentin interaction, which prevented to some degree the bond strength loss. In addition, the reduction in adhesive failures observed in the 2% DMSO resin indicates an improvement to some extent of the bonded interface integrity. Since DMSO has the ability to inhibit collagenases (Xu et al., 2004), a bonded interface with increased durability was formed due to reduced hydrolytic degradation of the collagen portion of the hybrid layer. In resins containing 4% and 10% DMSO, better collagenase inhibition by higher DMSO concentrations might have occurred on the expense of increased water sorption, solubility and drastic reduction of the polymer mechanical properties, invariably resulting in severe bond strength loss over time.

Microtensile bond strength results after two years of aging are in accordance with previous studies showing that degradation of the relatively hydrophilic adhesive interfaces after long-term aging is practically inevitable (Frassetto et al., 2015; Liu et al., 2011; Carvalho et al., 2012). Resin degradation is directly related to water sorption considering that hydrolytic attack on

ester linkages occur in the presence of water (Ferracane, 2006). Therefore, portions of the adhesive interface composed by loosely crosslinked polymers with higher water affinity represent potential sites for resin hydrolysis that may expedite the aging process due to increased water sorption. Regarding monomer conversion of adhesive resins, high degree of conversion of mono- and dimethacrylate resins may reduce the susceptibility of the polymerized adhesive layer and hybrid layer to hydrolysis, hypothetically improving dentin bonding durability. However, the 10% DMSO solvated resin, which presented the highest monomer conversion, produced the lowest immediate bond strength that was dramatically affected by aging in artificial saliva. Impairment of the mechanical properties and increase in water sorption/solubility in the resin with high DMSO content certainly contribute to higher bond strength loss over time. Therefore, DMSO incorporation in adhesive resins aiming to reduce bond strength loss over time should be sufficient to decrease the viscosity of the comonomers and facilitate the displacement of water from dentin, but low enough not to increase water sorption and solubility or to reduce polymer mechanical strength. Even though the inclusion of a single resin blend constitutes a shortcoming in the present study, it is safe to assume based on the regression analyzes that incorporation of approximately 1.6% DMSO in BisGMA/HEMA resin blends should produce promising results for future applications. More studies are required to find the ideal comonomer-solvent blend to optimize DMSO effect on dentin bonding and to understand the effect of DMSO on polymer chain crosslinking according to the resin blend's hydrophilicity.

5. Conclusions

DMSO incorporation in hydrophilic resins is a critical step in formulating dental adhesives since the effect of DMSO on the mechanical/physical properties and dentin bond strength of experimental resins was concentration dependent. While monomer conversion increased with 10% DMSO, water sorption, solubility and the mechanical strength of the adhesive resins were significantly hampered above 4%, expediting bond strength loss after aging. Nevertheless, 2% DMSO in conjunction with ethanol demonstrated the best overall effect on dentin bond strength improving resin–dentin bond durability.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.jmbbm.2016.07.003>.

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